

v-Liver™ The Virtual Liver Project

Simulating Hepatic Tissue Lesions as Virtual Cellular Systems

TRAC 2009, 4/28/09



This work was reviewed by EPA and approved for publication but does not necessarily reflect official agency policy.

Outline

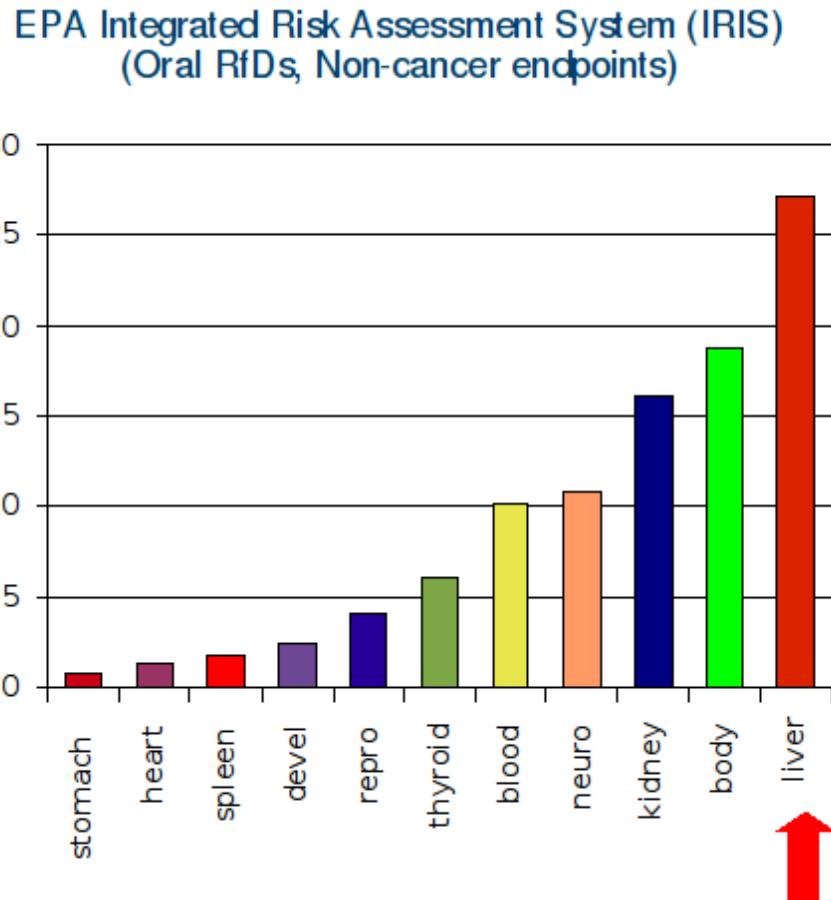
- Background & Motivation
- The need for Virtual Tissues
- The Virtual Liver Proof of Concept (PoC)
- Selecting chemicals using *in vitro* & *in vivo* data
- Tissue lesions simulation approach

Background

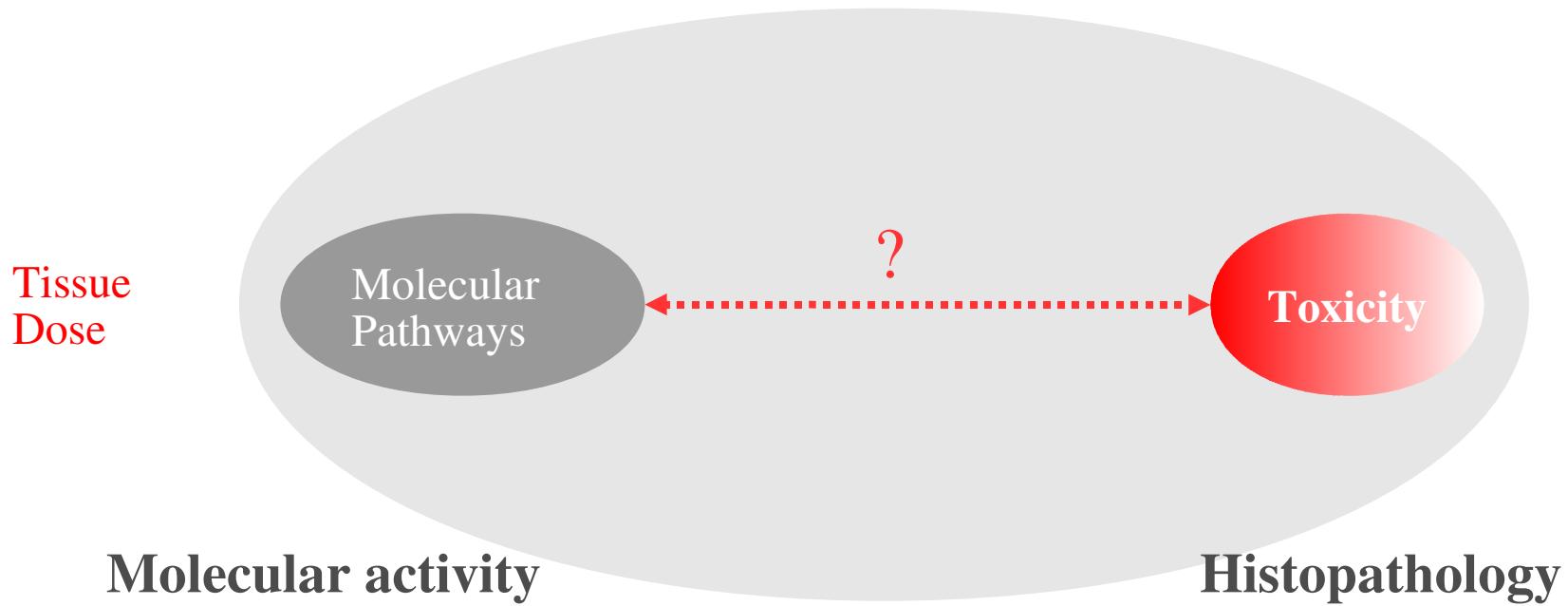
- ~10,000 HPV chemicals - little/no biological data !
- 2007 NRC - Toxicity Testing 21st Century
- 2008 EPA - Strategic Plan for Chemical Testing
 - Focus on “Toxicity pathways”
 - Novel *in vitro-in silico* extrapolation approaches
 - Reduce dependence on animals

Why Liver?

- **Primary** organ for environmental chemical **detoxification**
- **Most frequent** site of **adverse effects** (IRIS & ToxRefDB) in rodents – **relevant** to EPA
- **Large** amount of **available molecular** and **tissue** data
- **Unmet Need:** Extrapolation across doses, chemicals, species, populations



How to Predict Human Toxicity ?

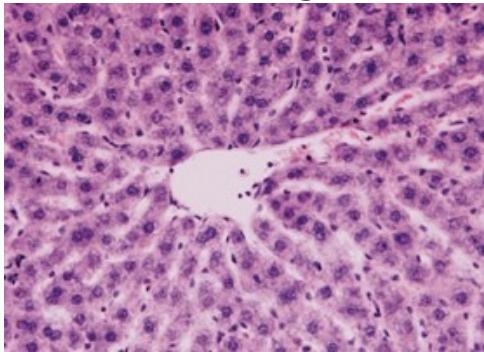


Prioritization: Molecular markers of adverse effects

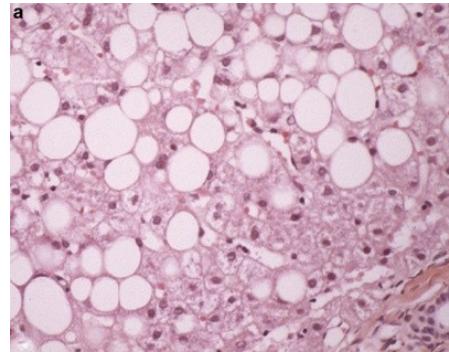
MOA / Dose-response: Need more biology

Toxicity: Tissue Lesions

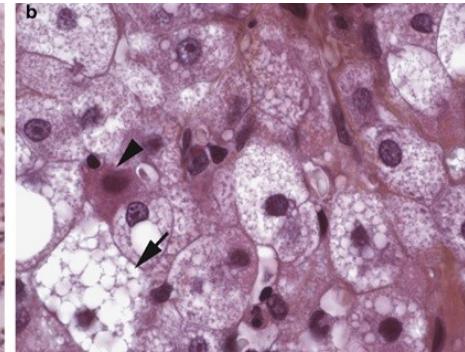
Swelling



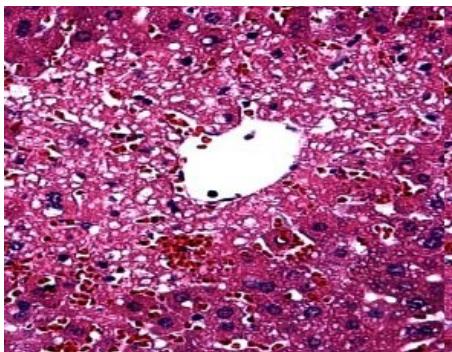
Steatosis, Macroses.



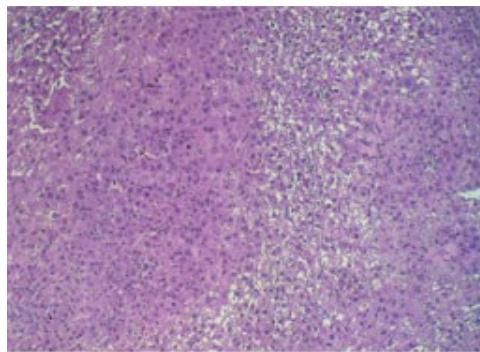
Steatosis, Microves.



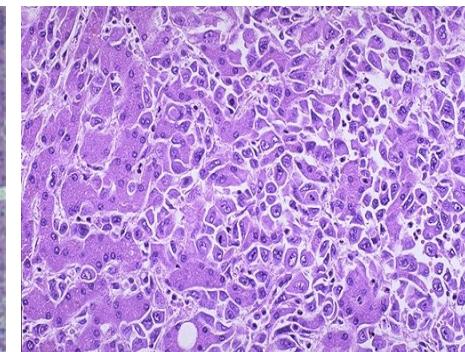
Necrosis



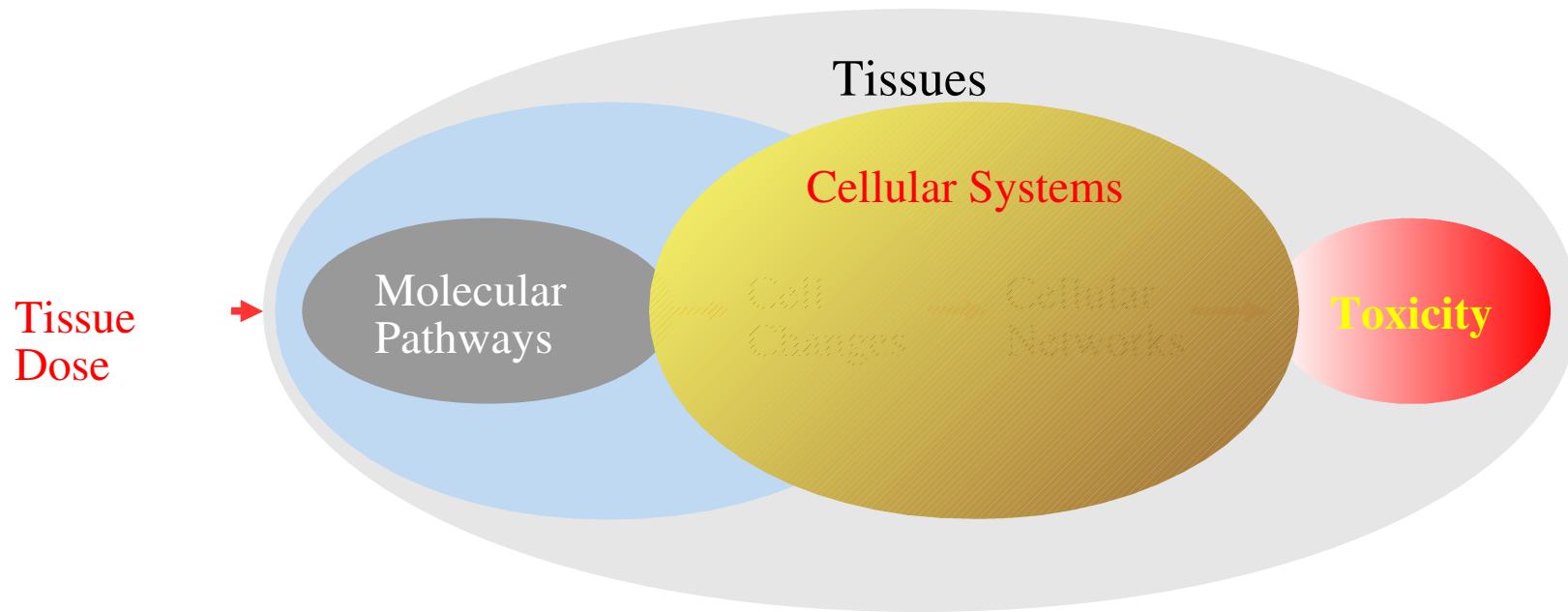
Hyperplasia



Carcinoma



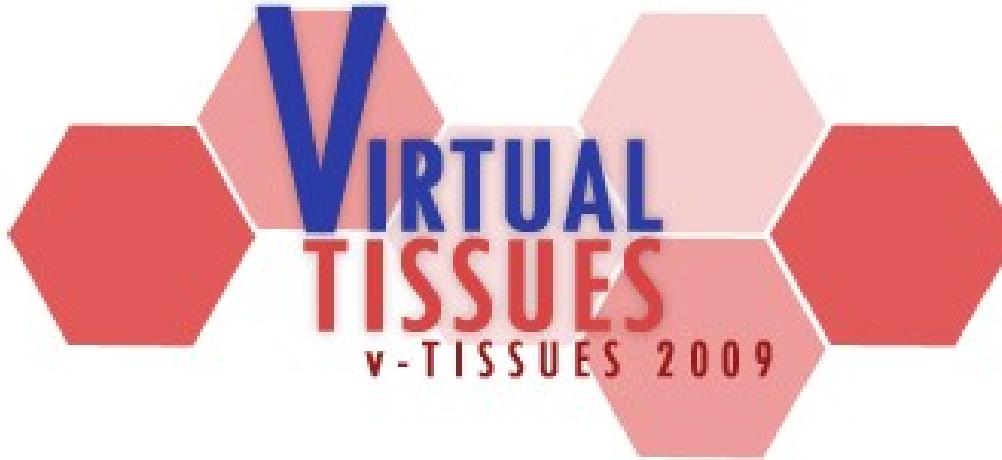
Tissue Lesions via Cellular System



Molecular pathways cause cellular changes

Cell changes propagated across tissues by cellular system

Relate molecular perturbations to lesions via cells



v-Tissues 2009

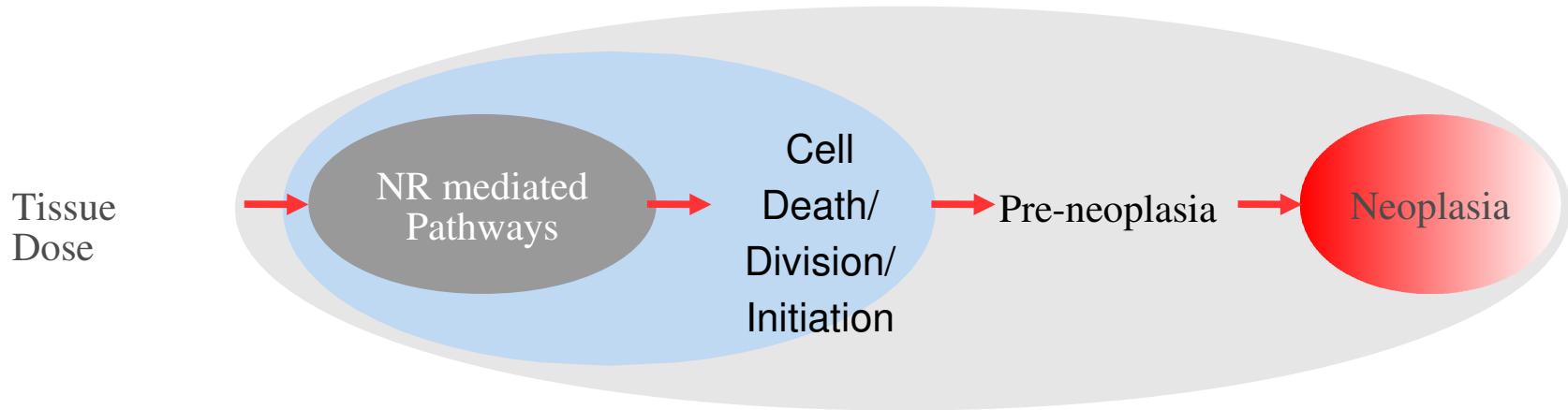
First International Workshop on Virtual Tissues

Hosted by National Center for Computational Toxicology

April 21-22 EPA Campus, RTP, NC

www.epa.gov/ncct/virtual_tissues

Key Science Questions



What nuclear receptor (NR) mediated pathways lead to cell necrosis, apoptosis and proliferation ?

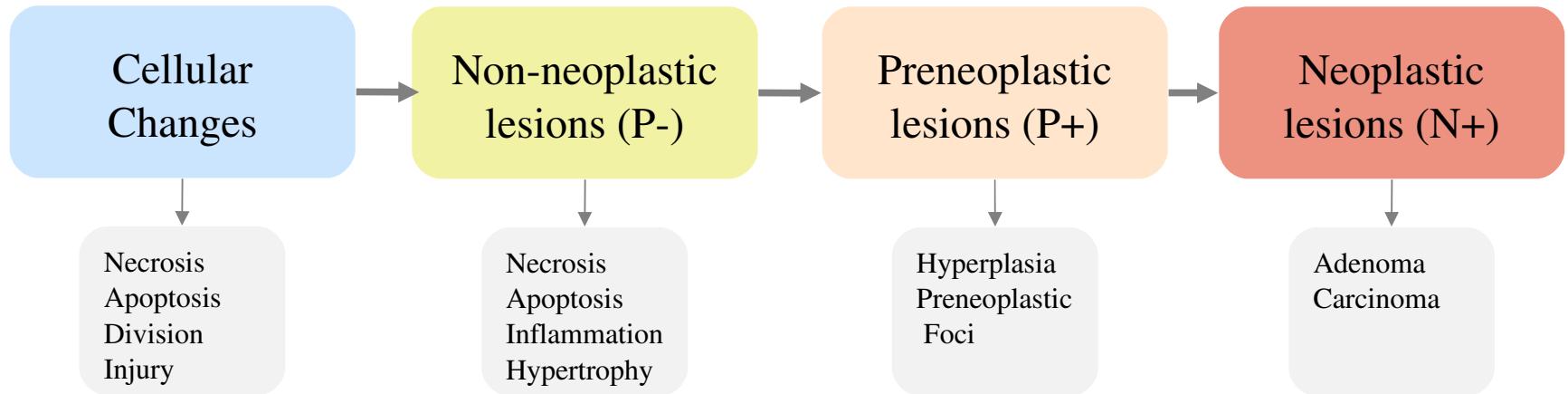
How do these cell alterations give rise to tissue lesions ?

Can we develop a framework to generate and test biological hypotheses for these questions ?

v-Liver™: Proof of Concept

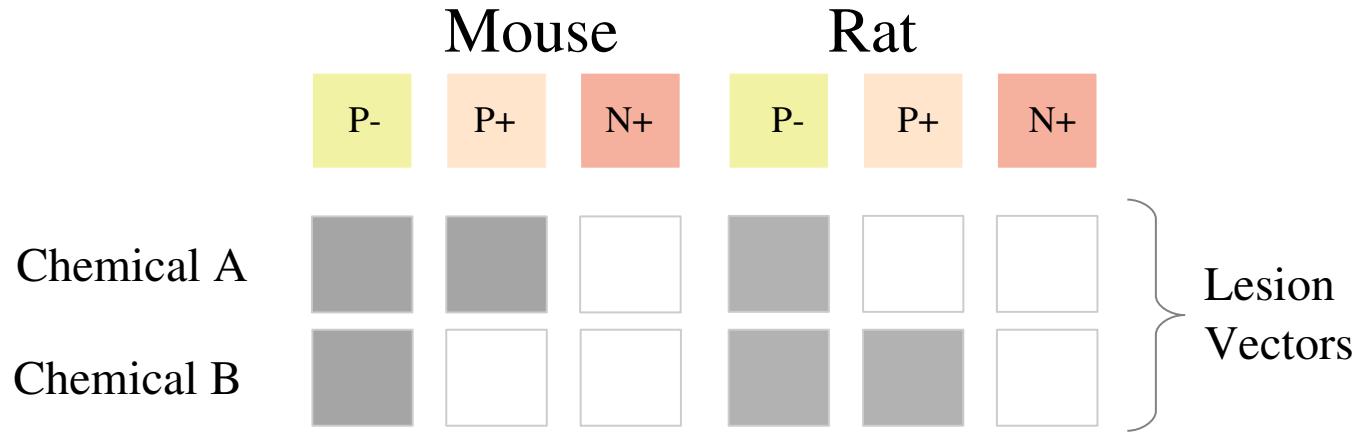
- A) Select environmental chemicals from ToxCast Phase I
- B) Integrate knowledge on key cellular events in hepatocarcinogenesis for PoC chemicals
- C) Develop tissue simulation platform: key molecular circuits, cell interactions and cell state changes
- D) Calibrate/Evaluate with *in vivo/in vitro* data using PoC chemicals

Lesion Progression in Cancer



- Lesions progress from subtle changes to neoplasms
- Histopathologic data available for intermediate stages
- Identify chemicals that cause these lesions across species

Data on Lesion Progression



- Data: ToxRefDB (Curated from DERs)
- Compare lesion progression across species and chemicals

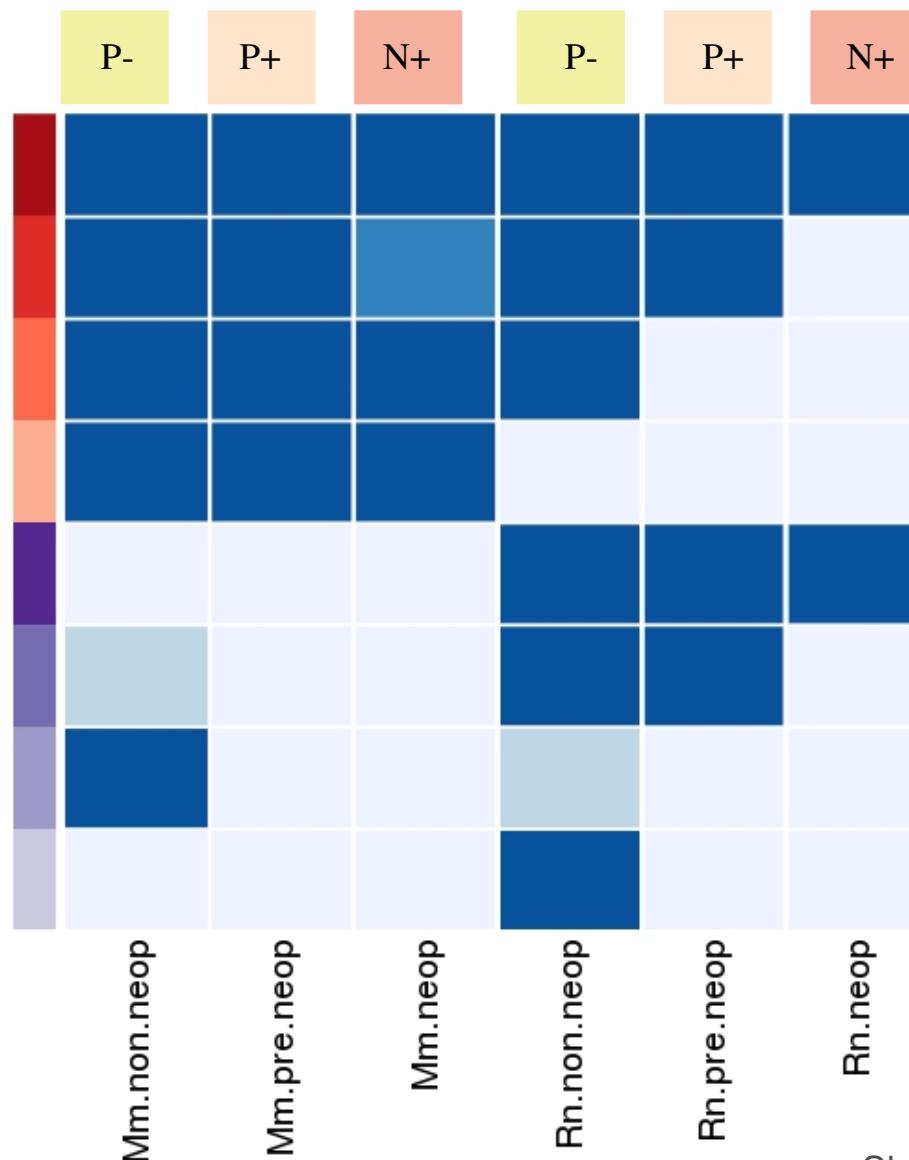
Cancer Lesion Progression

ToxRefDB:

220 Chemicals

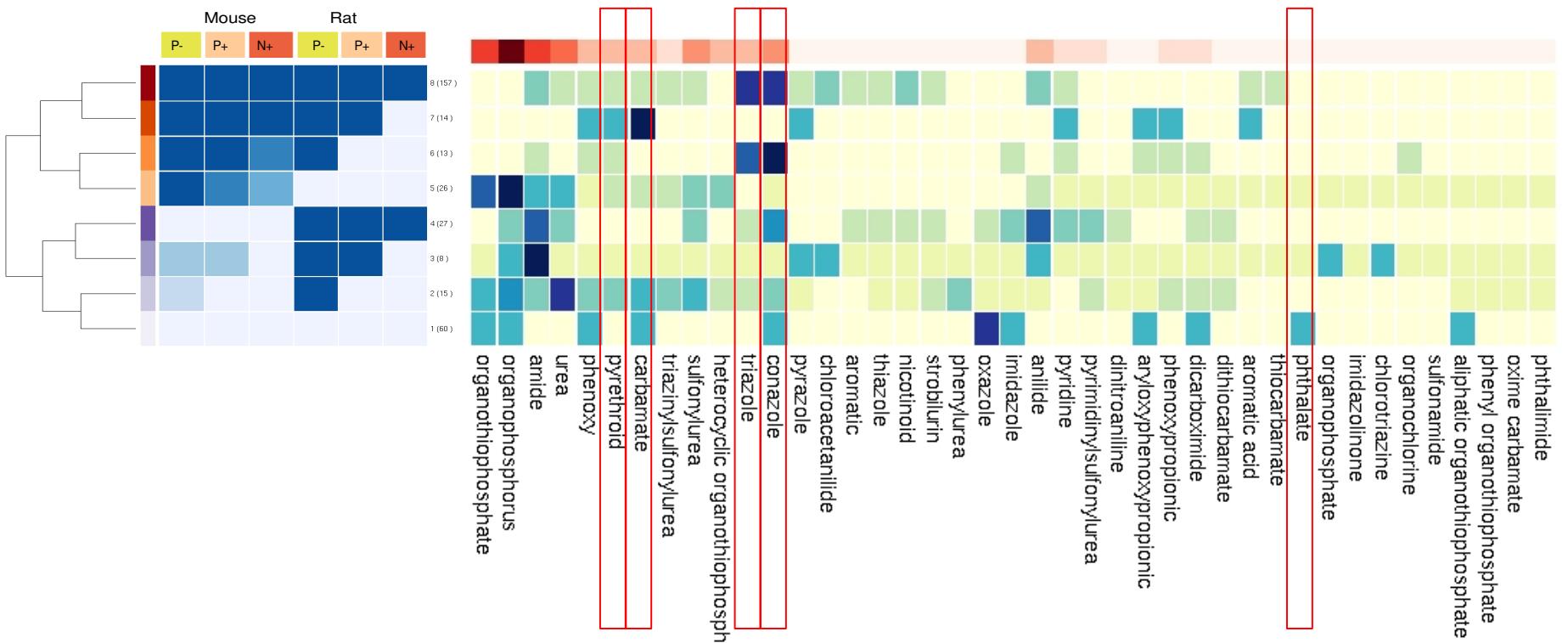
Rodent
testing studies

Detailed
histopathology



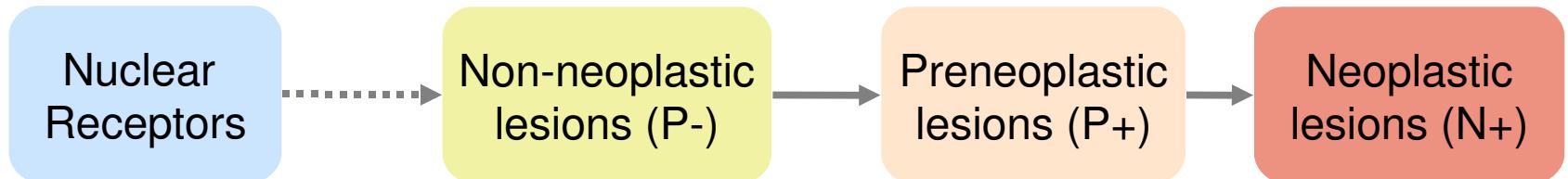
Groups of
Chemicals
Producing
Similar
Lesions

Chemical Classes and LP Groups



Shah et al. (in preparation)

Nuclear Receptors & Liver Cancer



- NR activity related to rodent liver cancer
- How is NR activity related to lesion progression classes ?
- Identify chemicals that activate NRs and lesions

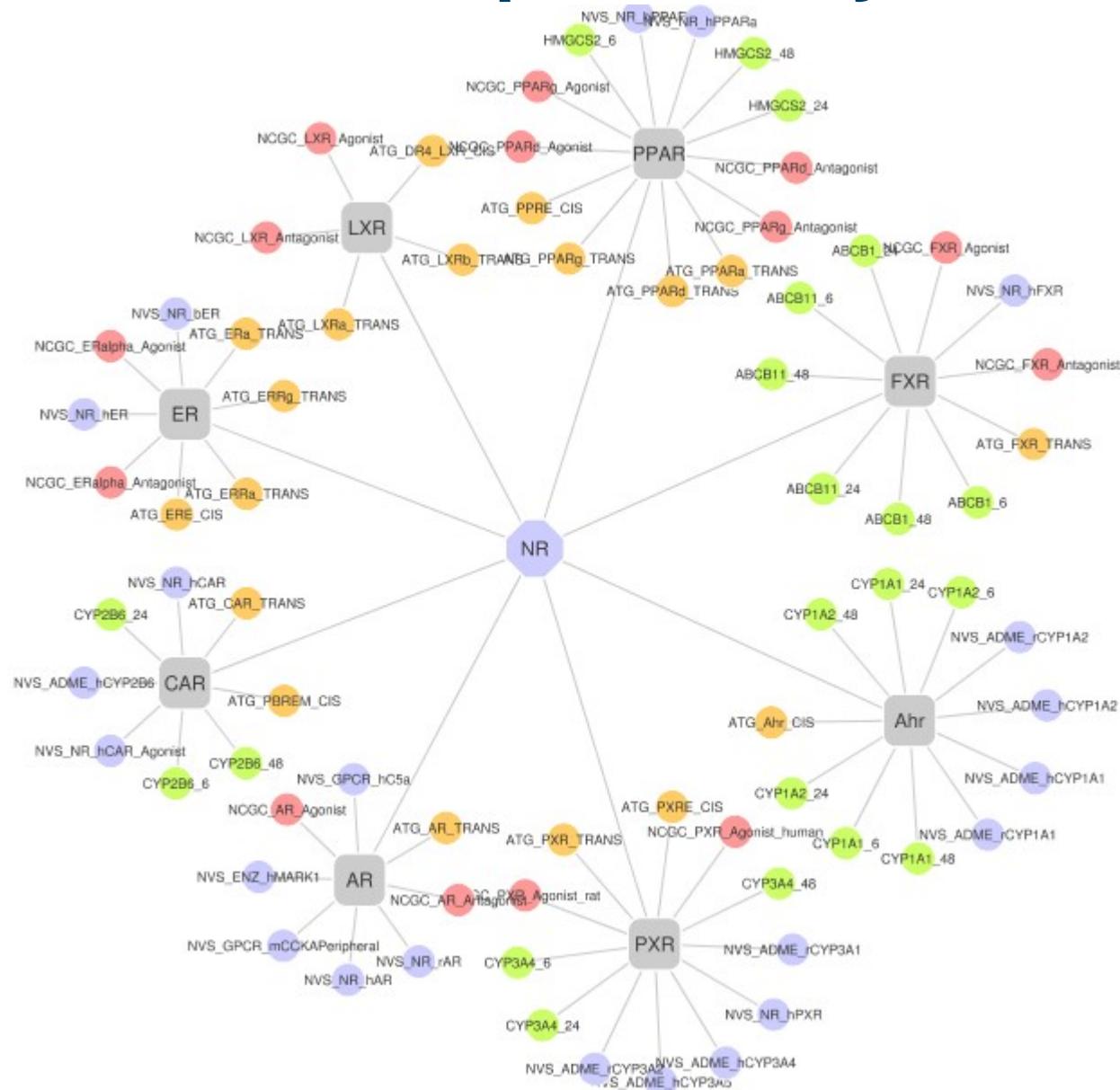
Nuclear Receptor Assays

HTS Assays

Multiple measures of activity

Gene regulation

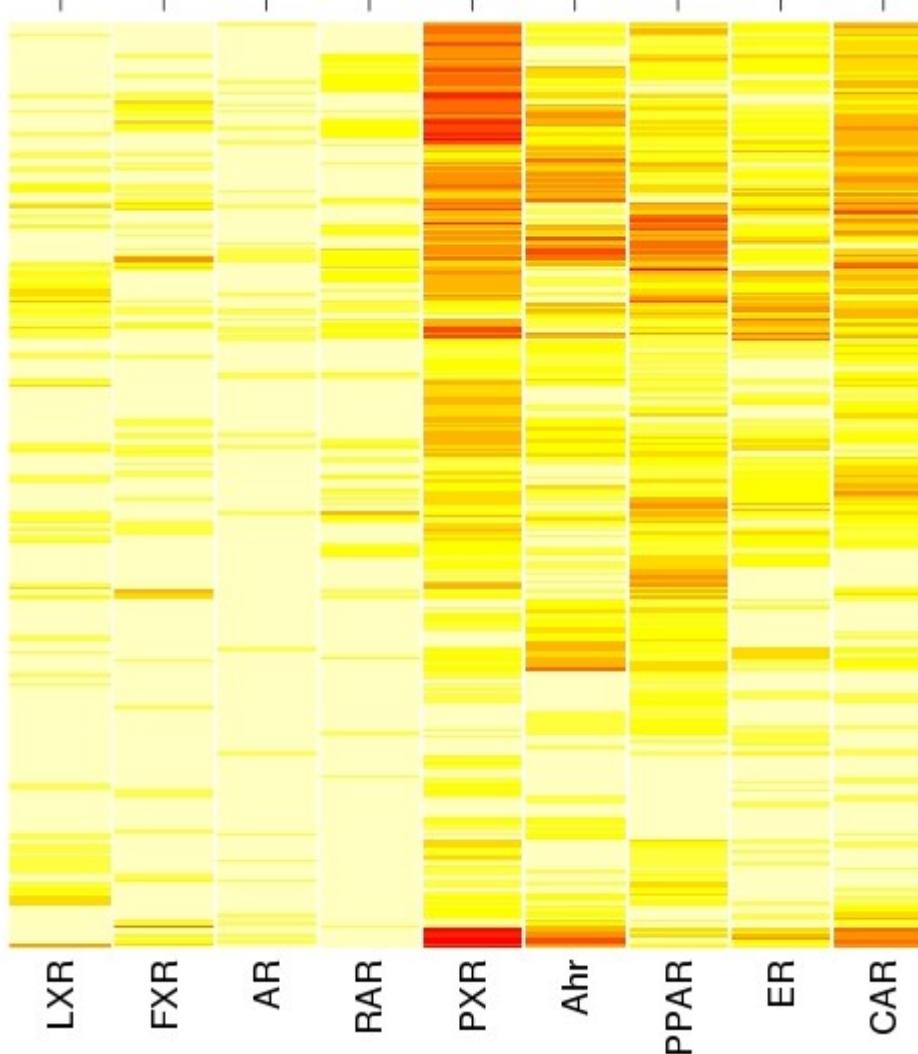
Ligand binding



Nuclear Receptor Activity

ToxCast™:
320 Chemicals

NR aggregate
Activity assays



Individual
Chemicals
NR
activity
profile

Lesions & NR activity

ToxCast:

309 Chemicals

600 Assays

Chronic Pathology

20 PoC Chemicals

Nuclear receptor +
Lesions: P-, P+, N+

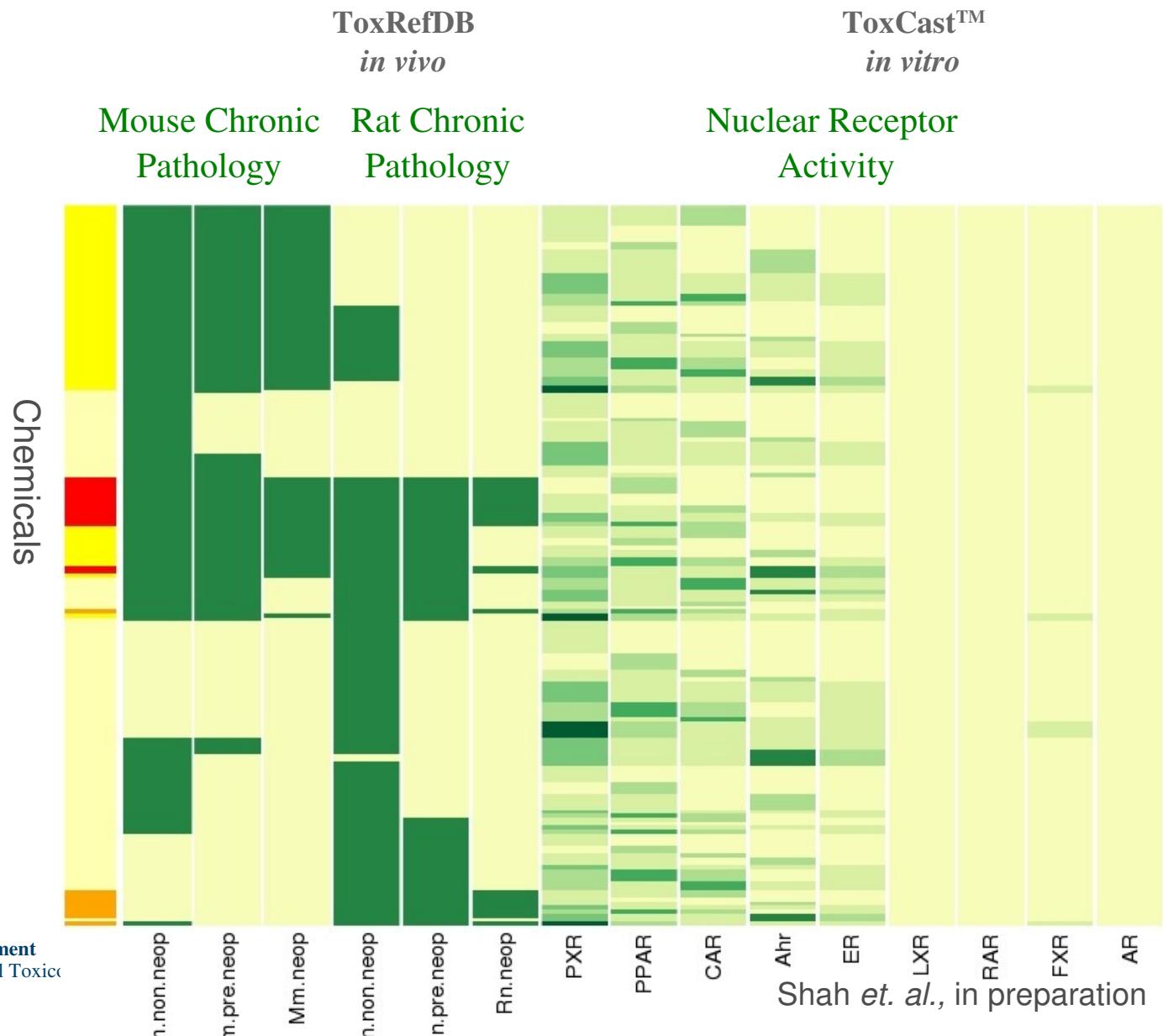
Candidates:

Conazoles

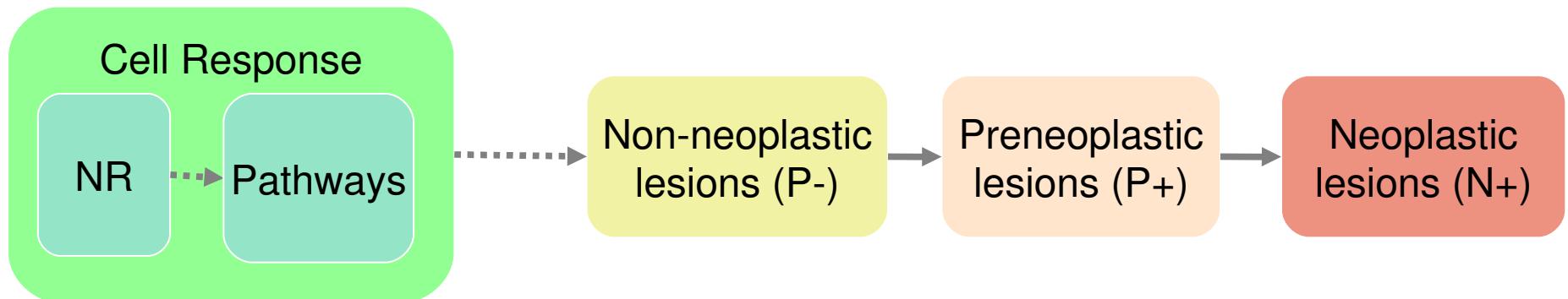
Pyrethroids

Phthalates

Perfluorinated



NR, Pathways and Cell Response

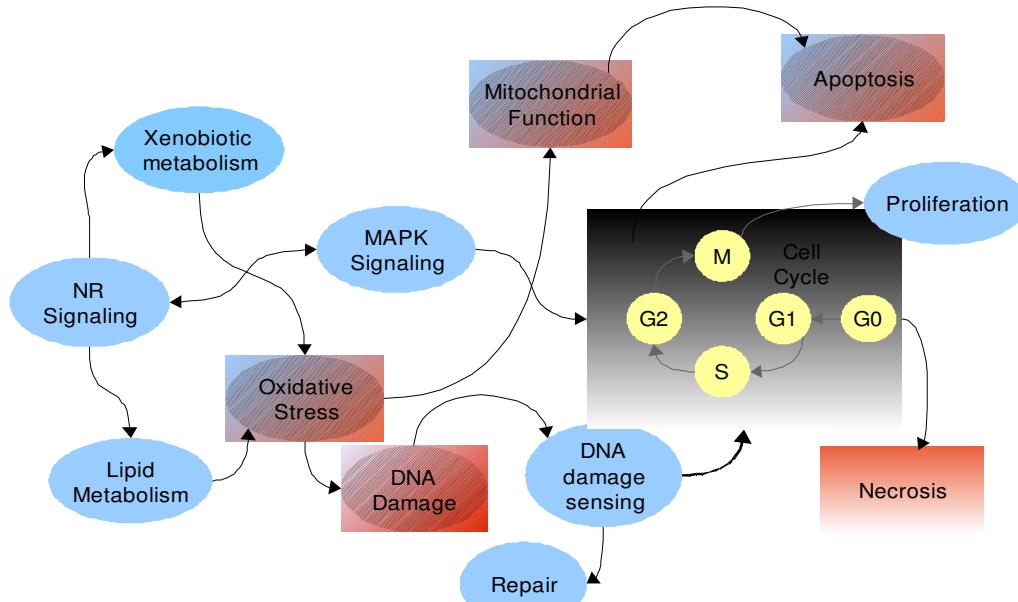


- Which NR-mediated pathways lead to cell proliferation / apoptosis ?
- How does persistent stimulation of NR cause non/pre/neoplastic lesions ?

Pathways & Cell Responses

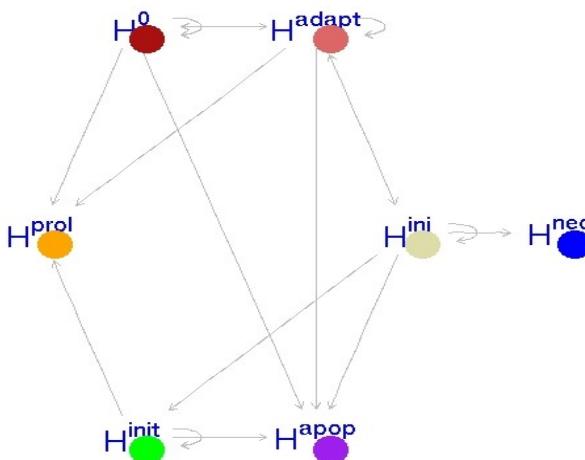
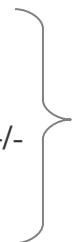
Mine literature, DBs
and prior data

(a) Cellular pathways &
interactions involved
in
death / division



(b) Cell inputs, states
and state transitions

Xenobiotic
Nutrients
Growth Factors+/-
Cell-contacts



Normal
Adaptive/Injury
Necrosis
Apoptosis
Proliferation
Initiation

Liver KB: Cytoscape Plugin



Slides

Cytoscape Desktop (Session: net-02-19-09.cys)

File Edit View Select Layout Plugins Help

Control Panel

Network \ VizMapper™ \ Filters \ Editor \

Network Nodes Edges

C-Mantic Network 24(0) 39(0)

C-Mantic Plug-In

Create Network

Use SPARQL queries to create Cytoscape networks.

Specify a data source

Define namespace prefixes

Enter and execute a query

Save Network

Save a Cytoscape network as an RDF file.

Save As...

Close

Panel

Overview

Rotate in Degrees:

0 90 180 270 360

Rotate Selected Nodes Only

Configure Database

Enter a SPARQL query service address.

URL:

Save

Specify Namespaces

Namespace	URI
owl	http://www.w3.org/2002/07/owl#
ti	http://www.epa.gov/ncct/liver-...
bpx	http://www.biopax.org/releases...
bp2	http://www.biopax.org/release/...
rdfs	http://www.w3.org/2000/01/rdf...
rdf	http://www.w3.org/1999/02/...
xsd	http://www.w3.org/2001/XMLSchema
tp	http://www.epa.gov/ncct/liver-...
ncl	http://pid.nci.nih.gov/biopax#
rx	http://www.reactome.org/biopax#
bn3	http://www.bionax.org/release/...

Apply QNames to Search Results

Add New Remove Selected Save

HepatoCyc-mol-int-1

Query

Query File:

```
construct
{?a ?b ?x .?x bp2:ID ?z .?x rdf:type ?y }
where
{ ?a ?b ?x .
?x rdf:type ?y .
?x bp2:ID ?z .
filter(str(?z)="200958")}
```

Execute Cancel

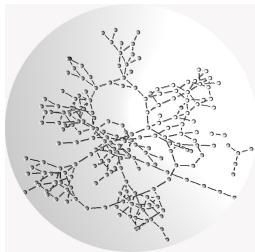
Subject Predicate Obj

Subject	Predicate	Obj
rx:UniProt_P20815_RecName__Full_Cyto...	bp2:SYNONYMS	CYP3A...
rx:UniProt_P20815_RecName__Full_Cyto...	bp2:SYNONYMS	"CYP3A5"^^<http://www.w3.org/...
rx:UniProt_P24462_RecName__Full_Cyto...	bp2:SYNONYMS	"CYP3A7"^^<http://www.w3.org/...
rx:UniProt_P24462_RecName__Full_Cyto...	bp2:SYNONYMS	"CYP3A7"^^<http://www.w3.org/...
rx:UniProt_P24462_RecName__Full_Cyto...	bp2:SYNONYMS	"CYP3A7"^^<http://www.w3.org/...

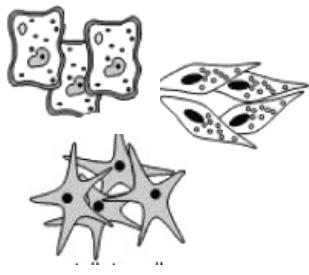
Node Attribute Browser Edge Attribute Browser Network Attribute Browser C-Mantic Results

Assembling the Components

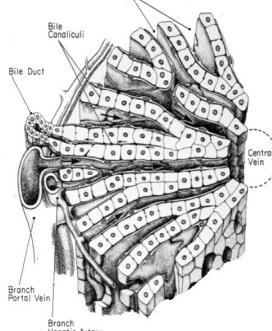
Biology



Molecular Pathways
To cell response



Cell junctions & signaling



Micro-circulation/
dosimetry

Off Nat
ment al Toxicology

Assumptions

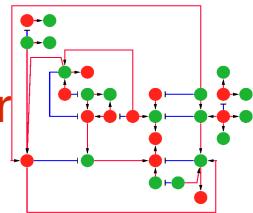
Key molecular events leading to discrete cell response

Subset of cell types, cell-cell junctions, communication

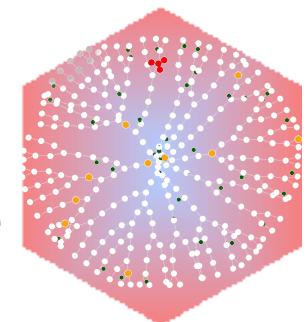
Two dimensional simplified flows

In silico

Molecular Logic & Decision

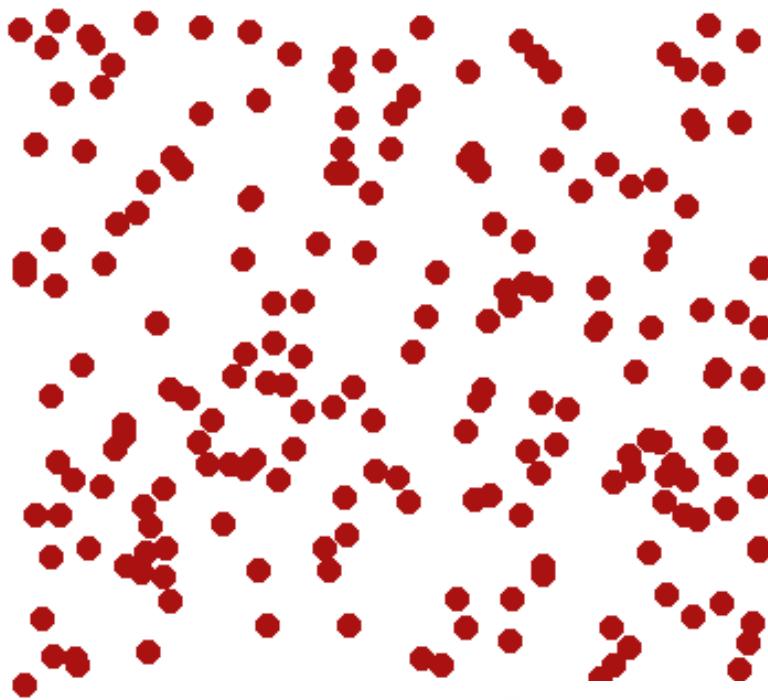


Cells as autonomous “Agents”

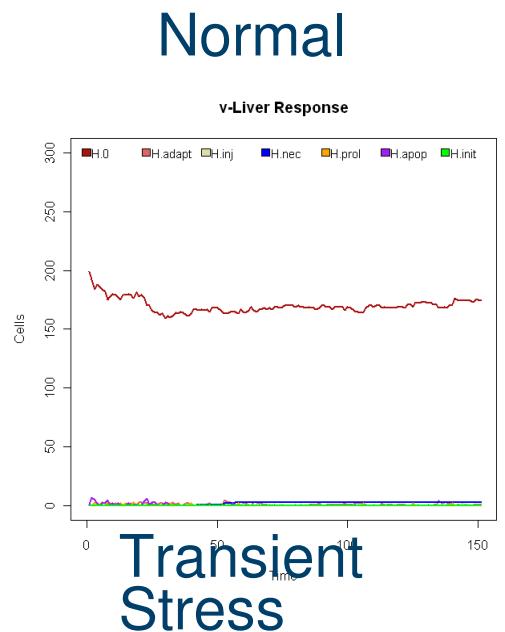
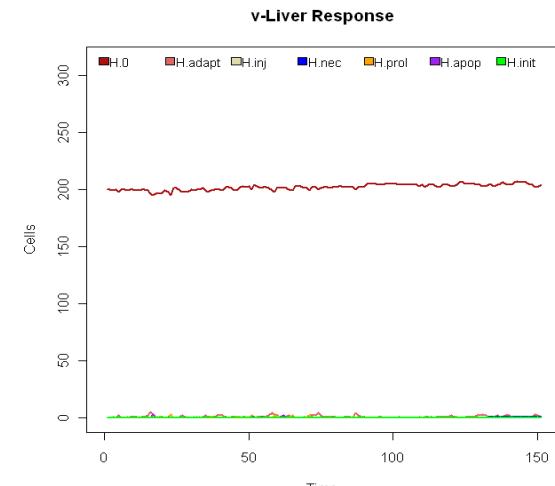
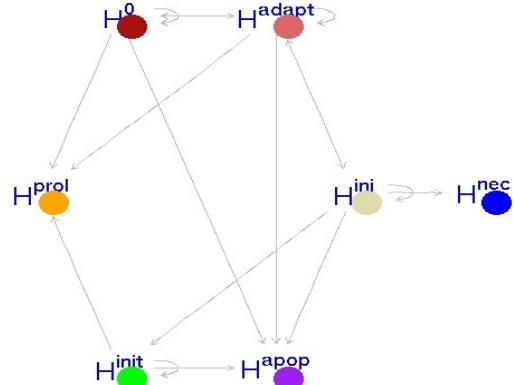


Virtual Lobule

Simulate *in vitro* Hepatocytes

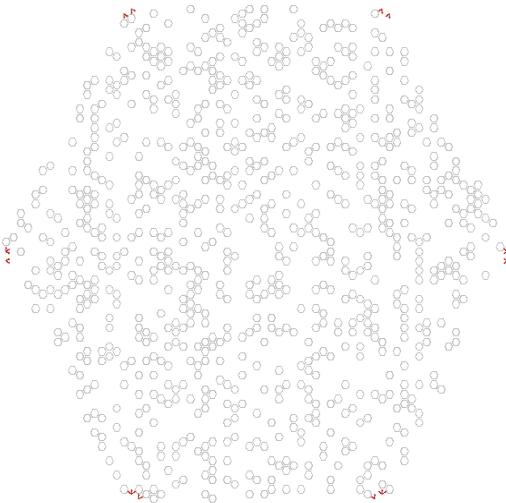


- Normal/Quiescent
- Stressed/Adaptive
- Stressed/Injured
- Necrotic
- Proliferative
- Apoptotic

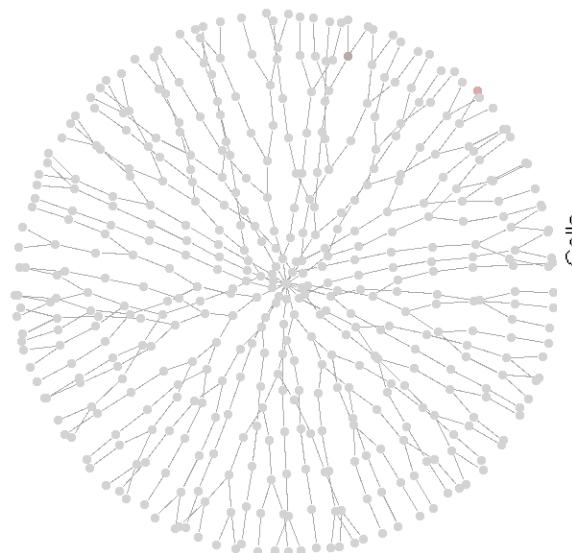


Simulating *in vivo* Effects

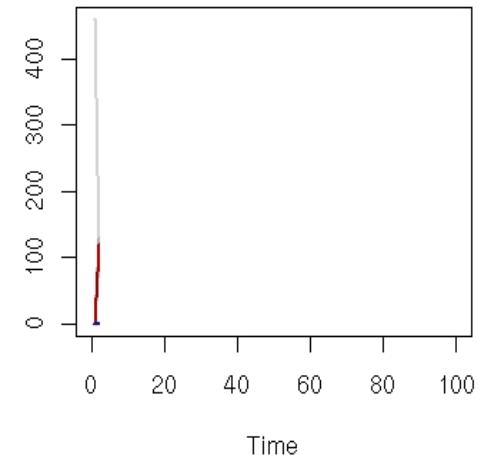
Microdosimetry



“Virtual Tissue”



Simulated Outcome



Multi-agent system:
spatial distribution of
agents with tight
junctions

Dynamics of multi-
agent system: temporal
changes in cell death/
proliferation

v-Liver: Milestones

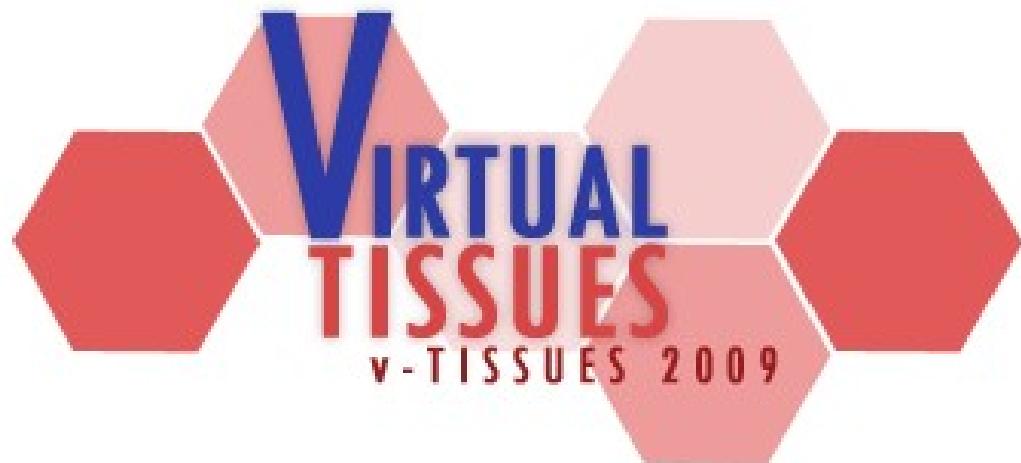
- FY 09
 - PoC chemicals: ~20 +/- hepatocarcinogens from ToxCast
 - KB: nuclear receptor-mediated molecular circuits with ToxCast / public domain data
 - Evaluate *in vitro* pathways for PoC chemicals
- FY 10
 - KB: Model nuclear receptor-mediated hyperplasia
 - Link with PBPK models for dosimetry
 - Evaluate *in vivo* predictions for PoC chemicals
- FY 11
 - Expand information between genomic variation and MOA
 - *in vivo* predictions for ToxCast Phase II chemicals / mixtures

Challenges

- Dealing with incomplete knowledge
- Using *in vitro* data in an *in vivo* context
- Dealing with time across molecular and cellular scales
- Evaluating *in vivo* predictions – how do we calibrate and evaluate tissue models ?

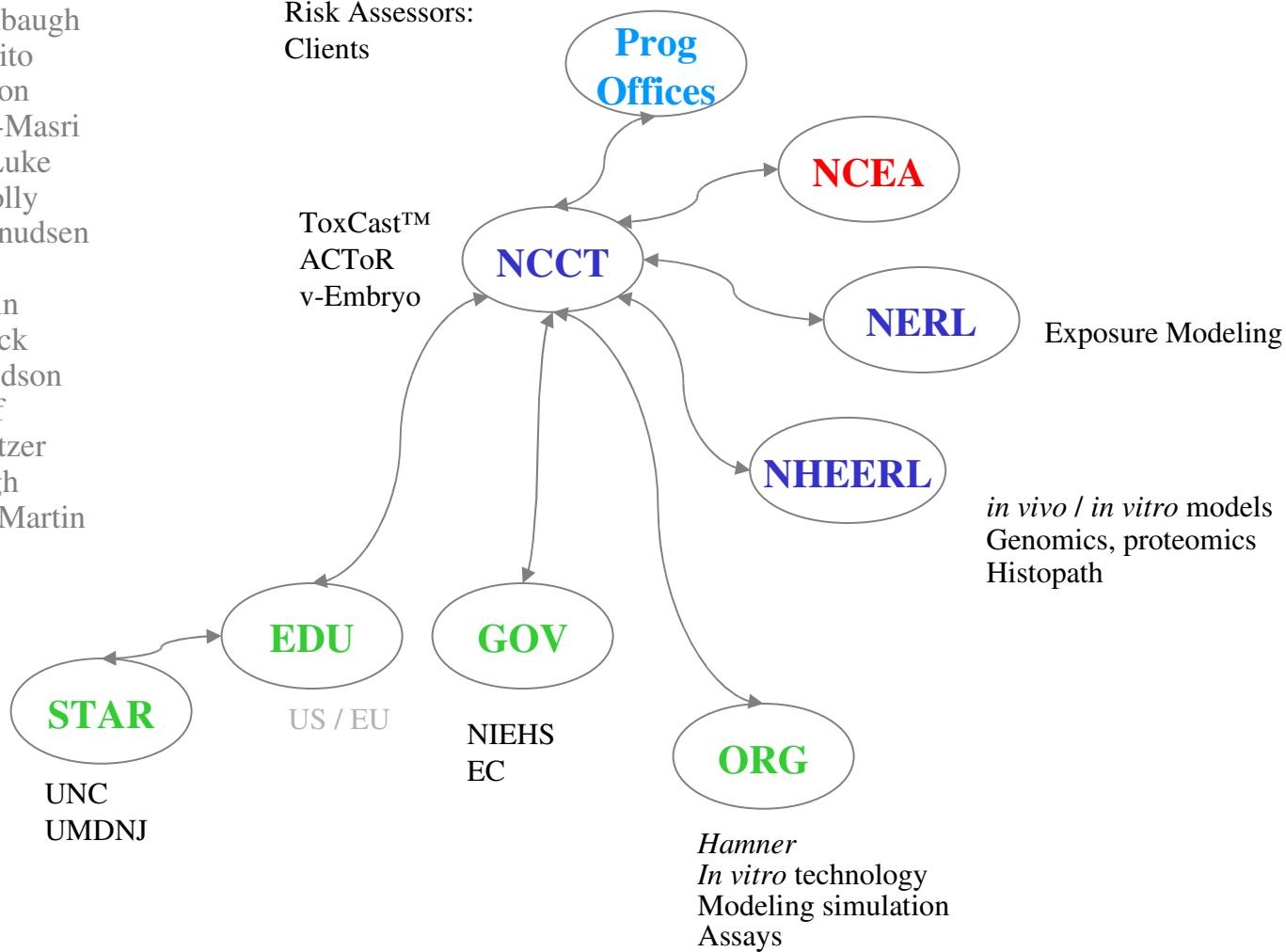
Summary

- Model tissues as “cellular systems”
- PoC: *in silico* platform to assess *in vivo* hepatic effects using *in vitro* data
- Focus on modeling lesions for ~20 NR+ hepatocarcinogens



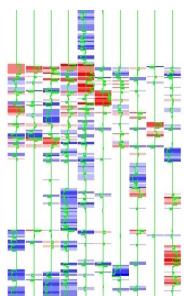
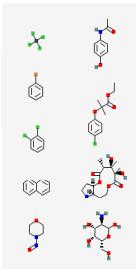
Multi-disciplinary Team: Cross-EPA/ORD & External

John Wambaugh
 Mike DeVito
 Chris Corton
 Hisham el-Masri
 Nicholas Luke
 Rory Connelly
 Thomas Knudsen
 David Dix
 Matt Martin
 Keith Houck
 Richard Judson
 David Reif
 Woody Setzer
 Amar Singh
 Lockheed Martin

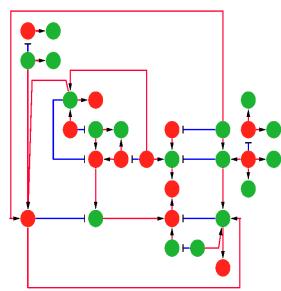


v-Liver™ Architecture

Assays



v-Liver Knowledgebase

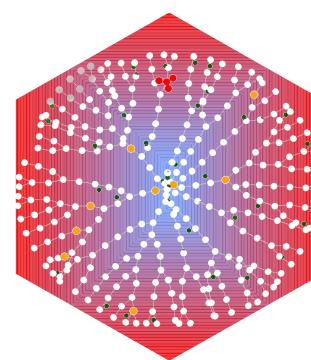


Molecular
Events

v-Liver Simulator

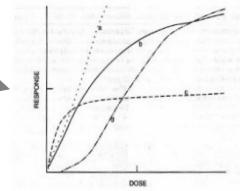
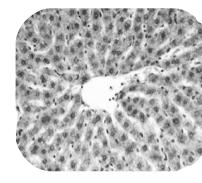


Cell-Cell
Events



Cell Sys. &
Blood Flow

Outcomes



Env.
Chems

ToxCast
HTS, HCS
ex vivo